



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

MICHEL GILBERT et al.

Application No.: 09/211,691

Filed: December 14, 1998

For: FUSION PROTEINS FOR USE IN  
ENZYMATIC SYNTHESIS OF  
OLIGOSACCHARIDES

Customer No.: 20350

Confirmation No. 9572

Examiner: Rao, Manjunath

Technology Center/Art Unit: 1652

DECLARATION OF DR. WARREN W.  
WAKARCHUK UNDER 37 C.F.R. §1.132

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Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

I, Warren W. Wakarchuk, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I, Dr. Wakarchuk, am currently Senior Researcher and Project Leader of the Glycosyltransferase project within the Immunochemistry section of the Institute for Biological Sciences of the National Research Council of Canada (NRC). I received my Master of Science degree in Microbiology from the University of British Columbia, Vancouver, British Columbia in 1984. I received my Ph.D. in Microbiology from the University of British Columbia, Vancouver, British Columbia in 1987. I was a post doctoral fellow in the Laboratory of Dr. Christoph Beck from 1987-1989. I was also a post-doctoral fellow in the laboratory of Dr. Stephan Withers. I joined the NRC in 1990 as a research associate in the laboratory of Dr.

**EXHIBIT A**

Makoto Yaguchi at the NRC laboratories in Ottawa as part of the Protein Engineering Network Centre of Excellence (PENCE). In 1994, I was appointed Associate Research Officer of the NRC. I became a Senior Research Officer at the NRC in 1999. I have been a member of the editorial board of the Journal of Biological Chemistry since July 2003. A copy of my curriculum vitae is attached hereto as Exhibit D.

3. The present invention is nucleic acids that encode fusion proteins comprising an  $\alpha$ -2,3-sialyltransferase that catalyzes the transfer of a sialic acid, from CMP-Neu5Ac, to an acceptor molecule; and a CMP-Neu5Ac synthetase that catalyzes the formation of CMP-Neu5Ac from Neu5Ac and CTP.

4. I am a named inventor on the above-referenced patent application. I have read and am familiar with the contents of this patent application. In addition, I have read the Office Action, dated October 2, 2003, received in the present case. It is my understanding that the Examiner is concerned that the specification does not provide sufficient structural description of the claimed invention. Specifically, the Examiner states that the specification does not describe any  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase fusion protein because the specification does not include a full length nucleic acid or amino acid sequence of such a fusion protein. The Examiner also states that the specification does not describe any other relevant structural characteristic of the claimed fusion proteins.

5. This declaration is provided to demonstrate that one of skill in the art would understand what was claimed based on the description of the invention in the specification. This declaration provides explanation of the disclosure of structure, thereby demonstrating that one of skill would recognize that the specification does provide a description the structure of at least three of the claimed nucleic acids encoding  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase fusion protein. The declaration also provides evidence of two other active fusion proteins comprising an  $\alpha$ -2,3-sialyltransferase and a CMP-Neu5Ac synthetase.

6. Example 1 of the specification provides instruction on making a fusion protein of *Neisseria*  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase enzymes. For example, the

specification provides reference to starting materials, provides specific primers to amplify the component nucleic acids that encode the fusion proteins, and provide cloning instructions to ligate the component PCR products. Three active  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase fusion proteins constructed using this method are disclosed in the application. In addition, Figure 1 and the accompanying legend provide a schematic view of one of the claimed  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase fusion proteins.

7. The application provides disclosure of a source for the sequence of a nucleic acid encoding a CMP-Neu5Ac synthetase protein and primers (e.g., SYNTM-F1 and SYNTM-R6) for amplification of the protein to make members of the claimed fusion protein genus. The specification provides reference to a source for a CMP-Neu5Ac synthetase nucleic acid at page 40, lines 4-5 (e.g., Gilbert *et al.*, *Biotech. Lett.* 19:417-420 (1997), submitted as Exhibit E); and to primers for amplifying the nucleic acid at page 40, lines 1-4. Gilbert *et al.* (1997) provides the amino acid sequence of a CMP-Neu5Ac synthetase protein and an accession number for a CMP-Neu5Ac synthetase nucleic acid, (e.g., U60146, submitted as Exhibit F), and those of skill would be able to identify a CMP-Neu5Ac synthetase nucleic acid based on that reference. Gilbert *et al.* (1997) also provides primers for amplification of the CMP-Neu5Ac synthetase nucleic acids from a *Neisseria* chromosome, e.g., SYNTM-F1 and SYNTM-R2. See, e.g., Gilbert *et al.* (1997) at page 418. The sequence of SYNTM-F1 is also disclosed in the application and is identical to the sequence in Gilbert *et al.* (1997). (See, e.g., specification at page 40, lines 1-2; and Gilbert *et al.* (1997), at page 417.) The SYNTM-R6 primer contains the final 27 bases of the SYNTM-R2 primer disclosed in Gilbert *et al.* (1997), and, at the 5' end, has an EcoRI site and nucleic acids that encode the linker sequence between the two enzymes. Thus, one of skill would recognize the sequence of CMP-Neu5Ac synthetase component of the disclosed fusion protein and the nucleic acids encoding that protein based on the disclosure of the specification and the references cited therein.

8. The application also provides disclosure of the sequence of the  $\alpha$ -2,3-sialyltransferase nucleic acid component disclosed. The specification provides description of the cloning of the  $\alpha$ -2,3-sialyltransferase component at page 40, lines 6-12. The  $\alpha$ -2,3-sialyltransferase was PCR amplified from a plasmid disclosed in the reference Gilbert *et al.*, J.

Biol. Chem. 271:28271-28276 (1996), submitted as Exhibit G. Gilbert *et al.* (1996) disclose amino acid sequences and accession numbers for  $\alpha$ -2,3-sialyltransferase nucleic acids from *Neisseria meningitidis* and from *Neisseria gonorrhoeae* (Accession numbers U60661 and U60664 are provided, submitted as Exhibits H and I) and primers for PCR amplification of those nucleic acids. The disclosed 5' primer is identical to nucleic acids at the 5' end of the *Neisseria gonorrhoeae*  $\alpha$ -2,3-sialyltransferase nucleic acid and includes an EcoRI site. The disclosed 3' primer is identical to the SIALM-18R primer of the reference with the addition of nucleic acids encoding a poly-His sequence, as required by the cloning scheme. Thus, one of skill would recognize the sequence of  $\alpha$ -2,3-sialyltransferase component of the disclosed fusion protein and the nucleic acids encoding that protein based on the disclosure of the specification and the references cited therein.

9. The application also provides disclosure of the fusion of the  $\alpha$ -2,3-sialyltransferase component and the CMP-Neu5Ac synthetase component of the encoded fusion protein. The  $\alpha$ -2,3-sialyltransferase nucleic acid and the CMP-Neu5Ac synthetase PCR amplification products were digested with appropriate restriction enzymes and ligated together using at an EcoRI site to form the fusion protein with a four amino acid linker (Gly-Gly-Gly-Ile) and His6 and c-Myc tags at the C-terminus of the  $\alpha$ -2,3-sialyltransferase enzyme. Specification at page 43, lines 20-22. Fusion proteins with 8 or 9 amino acid linkers were also constructed and each fusion protein had enzymatic activity. Specification at page 43, line 24 through page 44, line 5. The fusion protein with the 9 amino acid linker, pFus-01/2, had the highest activity and its structure is schematically depicted in Figure 1. Thus, one of skill would recognize the sequences of the disclosed  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase fusion proteins and the nucleic acids encoding those proteins based on the specification, the references cited therein, and Figure 1. The fusion nucleic acid and amino acid sequences described above are submitted as Exhibit B.

10. One of skill, on reading the above description of the structure of a nucleic acids encoding a  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase fusion protein, would recognize that other fusion proteins with the same general structure could be constructed. As an example, based on the disclosure in the specification, my laboratory has produced fusion proteins



using an  $\alpha$ -2,3-sialyltransferase from a different bacterial species. The sequences of a fusion proteins comprising *Campylobacter jejuni*  $\alpha$ -2,3-sialyltransferase and a *Neisseria* CMP-Neu5Ac synthetase are provided as Exhibit C. The fusions were made using either the full length *C. jejuni*  $\alpha$ -2,3-sialyltransferase (amino acids 1-430) or a truncated version of the *C. jejuni*  $\alpha$ -2,3-sialyltransferase (amino acids 1-328). Although the *C. jejuni*  $\alpha$ -2,3-sialyltransferase and the *Neisseria*  $\alpha$ -2,3-sialyltransferase share little identity at the sequence level, both fusion proteins had activity, i.e., both component proteins were active.

12. In view of the foregoing, it is my scientific opinion that one of skill in the art would recognize the structure of the three  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase fusion proteins disclosed in the specification, as well as the structure of nucleic acids that encode such proteins. In addition, one of skill in the art would recognize the class of  $\alpha$ -2,3-sialyltransferase/CMP-Neu5Ac synthetase fusion proteins based on the disclosure. The specification, therefore, describes the invention.

Date: Feb. 16, 2004

By: Warren W. Wakarchuk  
Warren W. Wakarchuk, Ph.D.

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